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NUTRITION REVIEWS

VOL. 17 AUGUST 1959 No. 8

PROTEIN AND AMINO ACID METABOLISM STUDIES WITH THE ISOLATED PERFUSED RAT LIVER

The technique of isolated liver perfusion is almost 100 years old in principle. Its direct approach to questions in hepatic physiology was early recognized, but technical restrictions peculiar to their times limited the applications of the early physiologists Ludwig, Bernard, and Schroeder. Later, Embden, Dakin, Nielsen and Lundsgaard used dog and cat liver perfusions for periods up to two hours with considerable success.

In retrospect, the limited ability of some workers (O. A. Trowell, *J. Physiol.* **100,** *432 (1941)*) to achieve a stable preparation with continued functional performance analogous to that of the liver in the intact animal is referable to: (1) the use of aqueous media, such as Ringer's solution, in place of blood; (2) the lack of mechanical filters to remove tiny clots which quickly plug the hepatic circulatory tree; (3) the relative unavailability of inexpensive, nontoxic anticoagulants, such as heparin; and (4) attempts to reverse the normal direction of blood flow through the liver by forcing blood into the thoracic vena cava, through the liver, and back out the hepatic portal vein.

Although the liver was long suspected on clinical and experimental grounds (S. C. Madden and G. H. Whipple, *Physiol. Rev.* **20,** *194 (1940)*) of being important in plasma protein synthesis, direct experimental evidence of the dominant role of the liver in the biosynthesis of the plasma proteins was presented in 1950 (L. L. Miller, C. G. Bly, M. L. Watson, and W. F. Bale, *J. Exp. Med.* **94,** *431 (1951)*). This report described the apparatus for and technique of perfusion of the isolated intact rat liver, utilizing homologous, oxygenated, heparinized blood and lysine-6-C^{14}. Significant serial observations were made for more than six hours; it was found that the qualitative and quantitative responses of the liver were in accord with observations made in intact animals.

In brief, the data strongly supported the view that perfusion of the intact liver with oxygenated blood permits the liver itself to synthesize plasma proteins, to separate them from its own tissue proteins, and to contribute them to the circulating plasma in a manner closely approximating the physiological, as seen in intact normal animals. Cannulation of the bile duct, which is carried out in routine fashion as a part of the preparation, permitted continued secretion and collection of the bile which, at very least, afforded a continuous measure of the viability of the preparation. The apparatus is designed to permit the continuous infusion of substrates, hormones or drugs in the course of the experiments. Finally, the multi-lobed rat liver is anatomically peculiarly suitable for the removal of samples of liver tissue at intervals in the course of experiments without in any way interrupting the perfusion.

The general availability of isotopically labeled materials and modern microchemical techniques of biochemistry have greatly broadened the potential applications and implications of the isolated perfused rat liver technique, as demonstrated in studies of protein and amino acid metabolism over the past eight years.

Although the earliest experiments afforded direct and unequivocal evidence for the dominant role of the liver in the bio-

synthesis of the plasma proteins, as indicated by the incorporation of lysine-6-C^{14}, these results were rendered more definitive by the application of preparative electrophoresis (H. G. Kunkel and R. J. Slater, *Proc. Soc. Exp. Biol. Med.* **80,** *42* (*1952*)) to plasma specimens from: (1) intact rats; (2) isolated, perfused rat livers; and (3) the eviscerated surviving rat (L. L. Miller and W. F. Bale, *J. Exp. Med.* **99,** *125* (*1954*) and L. L. Miller, C. G. Bly and W. F. Bale, *Ibid.* **99,** *133* (*1954*)). The isolated perfused liver, it was found, simulated the intact rat in the qualitative and quantitative pattern of oxidation of the carbon^{14} labeled amino acid to CO_2, and in the incorporation of the labeled lysine into *all plasma protein fractions with the exception of the gamma globulins.* The contribution of the non-hepatic tissues to the plasma proteins, first found grossly associated with the globulins, was later, by application of the above techniques, particularly to plasma from the eviscerated surviving rat, found associated almost *exclusively with the gamma globulins.* These observations have been amply borne out by a variety of experimental evidence implicating the lymphoid and plasma cells, but not the normal liver, in gamma globulin production.

Of equal and related interest is the demonstrated capacity of the isolated perfused liver to synthesize plasma lipids (K. I. Altman, L. L. Miller and C. G. Bly, *Arch. Biochem.* **31,** *329* (*1951*)) from carbon^{14} acetate, and the unequivocal responses to insulin of the isolated perfused liver with enhanced carbohydrate uptake and enhanced lipogenesis (D. E. Haft and L. L. Miller, *Am. J. Physiol.* **192,** *33* (*1958*) and **193,** *469* (*1958*)). The use of isotopes with the perfused liver technique is, as yet, virtually unexplored and unexploited by those interested in a direct study of the effects of various factors on hepatic lipoprotein production. Similarly, the factors affecting the production of the 'acute phase' proteins, fibrinogen and the alpha-2-globulins, should be readily evaluated by utilizing isotopically labeled metabolites in rat liver perfusion studies.

Urea production by the isolated perfused liver in the absence of added amino acid substrate proceeds in linear fashion, with the blood amino acid level remaining relatively constant. This continuing endogenous urea production, on the basis of C^{14} labeling experiments, is clearly derived from both plasma and liver proteins and quantitatively corresponds to most of the urinary nitrogen which would be lost by an intact animal corresponding to the liver donor's size (L. L. Miller, W. T. Burke and D. E. Haft, *Fed. Proc.* **14,** *707* (*1955*). In the same study, a clear cut, large protein sparing action of glucose and fructose was demonstrated in the normal perfused liver. When livers from alloxan diabetic animals were perfused, they were found (D. E. Haft and L. L. Miller, *loc. cit.*) to carry on protein catabolism at an enhanced pace. This abnormally high protein catabolism failed to respond either to insulin or to increased glucose or fructose uptake. A recent study (M. Green and L. L. Miller, *Fed. Proc.* **18,** *237* (*1959*)), makes use of leucine-C^{14} labeled liver and plasma proteins and presents evidence for the contribution of both the liver and plasma proteins to the enhanced protein catabolism of the alloxan diabetic liver.

The overall nitrogen metabolism of an added nutritionally complete mixture of 18 amino acids was studied in liver perfusions carried out in the absence and presence of glucose and fructose supplements (L. L. Miller, W. T. Burke and D. E. Haft, *loc. cit*). The latter reveals glucose to be more highly efficient than fructose in sparing nitrogen of the isolated perfused liver system. This was true although fructose supplements led to more enhanced liver glycogen levels. Thus measurement of net amino acid nitrogen disappearance, and urea nitrogen produced, permits an estimate of nitrogen balance in the isolated perfused

liver system. Positive nitrogen balance, connoting a net synthesis of protein, has been observed in this system (L. L. Miller, W. T. Burke and D. E. Haft, *Some Aspects of Amino Acid Supplementation, p. 44*, Rutgers University Press, 1955) only when continuous infusion of growth hormone and insulin was made. Depressed amino acid oxidation, diminished endogenous urea production, and enhanced incorporation of C^{14} labeled amino acids have been observed to occur in perfused experimental cirrhotic livers and in the perfused livers of experimental precancer produced by feeding 3 methyldimethylaminoazobenzene (W. T. Burke and L. L. Miller, *Cancer Research* **16,** *330 (1956)*; *Ibid*, **19,** *148 (1959)*).

The latter study reveals the quantitatively impressive role of glutamine nitrogen as a urea precursor. This was seen not only in normal livers but was observed to persist in precancerous livers under circumstances where urea production from the amino acids, arginine and aspartic acid, is significantly reduced (W. T. Burke and L. L. Miller, *Cancer Research, 1959*, in press). Such observations strongly support the existence of an important alternate pathway of ureogenesis from glutamine, such as suggested by Leuthardt and others. In contrast to glutamic acid, glutamine supplements were also observed to lead to diminished conversion of lysine-C^{14} to $C^{14}O_2$ and to enhanced incorporation into plasma and liver proteins.

The relative roles of the liver and of the non-hepatic tissues in the oxidation of 14 specific amino acids has been described in preliminary reports (L. L. Miller, W. T. Burke and D. E. Haft, *loc. cit*; L. L. Miller, *Fourth Int. Cong. Biochem. 12–29 (1958)*). The results of studies with isolated perfused livers and with the eviscerated surviving rat (with and without the liver left supplied by the hepatic artery) divide amino acids into two large groups; the first consists of those amino acids oxidized almost exclusively by the liver, such as arginine, histidine and lysine, and the second those amino acids oxidized as well by the non-hepatic tissues as by the liver, such as glutamic acid, aspartic acid and valine. The implications of these observations for the interpretation of intact animal studies with isotopically labeled amino acids and for an understanding of disturbances in amino acid metabolism in hepatic insufficiency remain to be evaluated.

The fact that the blood urea production continues while the blood amino acid level remains relatively constant, or increases slightly in the course of liver perfusions without added amino acids, led to an examination of the activity of the isolated perfused liver in the conversion of carbon^{14} labeled plasma proteins to $C^{14}O_2$. From such data, with several assumptions, minimum estimates of net plasma protein catabolism have been made (L. L. Miller, W. T. Burke and D. E. Haft, *loc. cit.*). Conservatively, it has been estimated that catabolism of the plasma proteins may account for at least 35 to 40 per cent of the endogenous urea nitrogen production. Because these studies were carried out with whole labeled plasma it is impossible to specifically designate the contribution of each of the several electrophoretic fractions; however, if the three to four fold greater extent of labeling of the α globulins (L. L. Miller and W. F. Bale, *J. Exp. Med.* **99,** *125 (1954)*) is any indication of the relative rate of degradation, then one may infer that the α globulins not only pick up much more carbon^{14} label in the process of synthesis, but also give up their label correspondingly more rapidly in the process of catabolism.

The role of the liver in the catabolism of iodine^{131} labeled plasma albumin has been studied in the isolated perfused rat liver (S. Cohen and A. H. Gordon, *Biochem. J.* **70,** *544 (1958)*). The extent to which trichloracetic acid soluble iodine^{131} appears in the circulating perfusate is used as a measure of catabolism. It was previously observed (A. H. Gordon, *Ibid.* **66,** *255 (1957)*) that

the perfused rat liver rapidly catabolized small proportions of altered or denatured protein molecules present in preparations of iodine^{131} labeled plasma proteins. T. Freeman, A. H. Gordon and J. H. Humphrey (*Brit. J. Exp. Path.* **39,** *459 (1958)*) have sought to evaluate the relative roles of the reticulo-endothelial cells and the hepatic parenchymal cells in the catabolic breakdown of native and heat denatured labeled proteins. The results of reticulo-endothelial blockade by carbon injection show diminished uptake and breakdown of heat denatured proteins. Native albumin, on the contrary, was taken up and broken down at a rate four to five times that observed in the normal liver untreated with carbon injection.

Further studies with the perfused rat liver promise a better understanding of the exact cellular sites and mechanisms of hepatic protein synthesis and catabolism. Thus far, such studies have established the role of the liver in plasma protein catabolism at both the macromolecular and amino acid levels. More soundly established quantitative aspects and the variations from the normal with experimental diseased states remain to be elaborated.

The ease with which the rat liver perfusion technique may be learned, its ready reproducibility, and its metabolic stability over periods of four to six hours, at least, recommend it as a useful and at times highly valuable approach to many problems in liver physiology and biochemistry of both normal and abnormal states.

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"LITERARY" OBESITY—THE PICKWICKIAN SYNDROME

In his descriptive style, Charles Dickens depicted a character who had many of the clinical features of a malady which accompanies extreme obesity. C. S. Burwell, E. D. Robin, R. D. Whaley and A. G. Bickelmann (*Am. J. Med.* **21,** *811 (1956)*) published a humorous yet scholarly account of this disorder and applied the term "Pickwickian Syndrome" to these patients. Nosologically this condition might be called "alveolar hypoventilation and polycythemia in extremely obese patients", but the descriptive name has gained popular acceptance and is here to stay.

True, many words of ridicule and jest (even of indignation) have appeared in the pages of the *New Engl. J. Med.* (**257,** *1238 (1957)*; **258,** *507, 1020 (1958)*; **259,** *34 (1958)*), in the *Brit. Med. J.* (**2,** *964 (1958)*), and elsewhere. Actually Mr. Pickwick, although corpulent, was an agile industrious and athletic man who had none of the symptoms of this illness. It was the servant boy of his acquaintance, Mr. Wardle (known as "Fat Joe"), who possessed these traits. Indeed, he was described as sleeping at every opportunity and it was said that he "goes on errands fast asleep and snores as he waits on tables".

Perhaps it was fortunate that such a name was given to this disorder, for many fine studies have appeared since its publication, (G. A. Lillington, M. W. Anderson and R. O. Brandenburg, *Diseases of the Chest* **32,** *1 (1957)*; H. E. Estes, Jr., H. O. Sieker, H. D. McIntosh and G. A. Kelser *Circulation* **16,** *179 (1957)*; T. Richter, J. R. West and A. P. Fishman, *New Engl. J. Med.* **256,** *1165 (1957)*; G. N. Bedell, W. R. Wilson and P. M. Seebohm, *J. Clin. Invest.* **37,** *1049 (1958)*). These have familiarized us with the salient features; marked obesity with a short stature, somnolence, twitching, cyanosis, periodic respirations, secondary polycythemia, right ventricular hypertrophy and right ventricular failure. Furthermore,

it has been shown that obesity leads to faulty ventilation resulting in a high alveolar concentration of carbon dioxide and secondary hypercapnia with hypoxia. Further depression of the respiratory center with somnolence and twitching result from these.

A typical case is described by R. M. Cherniack (*Canad. Med. Assn. J.* **80,** *613 (1959)*). This was a 51-year-old farmer, five feet four inches tall and massively obese. Aside from moderate exertional dyspnea, he had been well until he began to note extreme fatigue followed by somnolence. His wife found it necessary to awaken him often each day. He developed headaches and was too tired to perform much of his work as a farmer. One day while harvesting his fields he went to sleep but could not be awakened. He was taken to a hospital where he was treated with oxygen. Examination of his blood revealed his hemoglobin to be 19.6 g. per cent and his erythrocyte count to be 6.5 million. He recovered following phlebotomy of two liters of blood.

Two years later he returned in congestive cardiac failure. He was cyanotic, dyspneic and confused. His heart was grossly enlarged and his ventilatory function was markedly reduced. After treatment of the failure, he was made to reduce from 225 pounds to 162 pounds with good recovery.

This case exemplifies the syndrome, a gradually progressive impairment of pulmonary ventilation which leads insidiously to depression of the central nervous system, cardiac failure and death. The presence of polycythemia, although attributed to hypoxia, may be related to other factors. P. Szenas and C. J. Pattee present evidence of increased adrenal cortical activity in obese patients (*J. Clin. Endocrin. & Metab.* **19,** *344 (1959)*). They found that urinary excretion of 17-hydroxycorticosteroids was increased in 16 obese subjects and that their serum concentrations of this steroid were low. They interpreted this as evidence for rapid production and utilization. Perhaps there is a relationship between the "Cushing's like" features of extreme obesity and the adrenal cortical function of these patients.

VITAMIN K DEFICIENCY IN NEWBORN INFANTS

In recent years, there has been a relative disappearance of hemorrhagic disease of the newborn in the United States and Western Europe. However there has been a lack of clear-cut evidence that prophylactic vitamin K administration to either mothers or infants would prevent hemorrhagic disease, or that vitamin K deficiency was associated with the specific clotting disturbances ordinarily observed in the newborn period (H. Dam H. Dyggve, H. Larsen and P. Plum, *Advances in Pediatrics* **5,** *129 (1952)*). This has led authorities to state that the value of routine prophylactic vitamin K administration was not clearly established (*Recommended Dietary Allowances, National Research Council, Publication 589 (1958)*). Critical studies of the problem can be made only in a population in which hemorrhagic disease of the newborn occurs relatively frequently, and where investigators are equipped with the latest techniques for evaluating a complicated coagulation disorder.

A. J. Aballi, V. L. Banus, S. de Lamerens and S. Rozengvaig (*J. Dis. Child.* **97,** *524 (1959)*) have reported coagulation studies in 26 cases of hemorrhagic disease of the newborn, occurring in a 32 month period, on the maternity service of the General Calixto Garcia Hospital in Havana. This represented an incidence of one case for every 300 deliveries.

Criteria used for selecting the cases were

strict. All cases of bleeding in prematures below 2,250 g. were excluded, as were cases of severe trauma, accident or obvious disease. The mothers of these infants came from the poorest classes and had received no vitamin K prophylaxis during this study.

The clinical manifestations consisted of blood in the vomitus, the stool or bleeding from the nose in the majority of cases. The hemorrhage was noted some hours following birth (at birth in two cases only) and before 48 hours of age in 19 of the 26 cases. All of the patients were treated with vitamin K except for one receiving only transfusions. Three of the patients failed to respond; two of these had been subjected to severe anoxia during labor and the third one had severe fatty infiltration of the liver. All three of these patients died.

Studies of the clotting mechanism were carried out in 17 cases using modern techniques. The findings in these cases were very similar to those of the so-called physiological hypoprothrombinemia of the newborn, but were much more severe. For example, the one-stage prothrombin time, which was 89 ± 11 per cent in adult controls and 44 ± 27 per cent in full-term infants, averaged 4.8 per cent in the infants with hemorrhagic disease. Likewise, factor VII (proconvertin-stable factor), which was reduced in normal newborns to 32 per cent of normal adult controls, was 0.5 per cent in the infants with hemorrhagic disease. There is no question that the severity of the clotting defects in these infants was well below the physiological drop in these two factors which occurs in normal infants. The reduction in the one-stage prothrombin time was due to a reduction of true prothrombin. The clotting time was prolonged but the bleeding time was within normal limits.

There was also a marked alteration in the first stage of coagulation, with a marked decrease in thromboplastic activity of the serum due to several factors. These include the plasma thromboplastic component (PTC) and an unidentified thromboplastic substance which might possibly be the Stuart-Prower factor. These authors point out that the clotting defect in the newborn with hemorrhagic disease is very similar to that produced by the prolonged use of coumarin drugs, which presumably act as vitamin K antagonists. Detailed studies in five infants failed to reveal any evidence of any anticoagulant materials in their blood.

The alterations described responded spectacularly to vitamin K administration with significant correction within six hours and return of the values, usually within 24 hours, to within the range found in normal newborn infants in the same population. Blood transfusions were used whenever the blood loss was severe, and it was pointed out that, in the face of massive bleeding, very large amounts must sometimes be given.

The cases of anoxia complicated by multiple hemorrhages were thought to constitute a separate entity. Because of the liver damage, they did not respond to vitamin K. Furthermore these infants had evidence of capillary damage, which was not seen in those with uncomplicated hemorrhagic disease.

These workers feel that the disappearance of hemorrhagic disease in many parts of the world in the last two decades is significantly related to vitamin K prophylaxis, and that statistical analyses in such a complicated situation are difficult to control. No matter what the interpretation is of other published data, it is quite apparent that in the population studied, the frequency of hemorrhagic disease in the absence of vitamin K prophylaxis or early feeding was alarmingly high. The response to treatment with vitamin K was equally dramatic.

This excellent demonstration, with clear-cut detailed evidence completely compatible with the hypothesis that these severe coagulation defects are due to vitamin K

deficiency, is an important contribution to our understanding of a deficiency disease now disappearing in the United States and Europe. It is clear that vitamin K deficiency in the newborn still occurs in certain populations and that its treatment and presumably its prevention can be effected by vitamin K administration.

VANADIUM INHIBITION OF CHOLESTEROL SYNTHESIS

On grounds of statistical association, the concentration of cholesterol in the serum has been linked to cardiac disease, particularly of the coronary type (*Nutrition Reviews* **13,** *138* (*1955*)). Whether this association is fortuitous or causative, it has evoked great interest in the metabolic functions of cholesterol and related lipids (*Ibid.* **13,** *8*, *44* (*1955*); **14,** *193* (*1956*); **15,** *1*, *17* (*1957*)).

Naturally enough, investigators have sought methods which could decrease the serum cholesterol, in hopes of lessening the frightful frequency of coronary artery disease in our "civilized" populations. These attempts have varied in their approach, effectiveness and complexity.

One approach which has been mentioned is through dietary elimination of animal fats and proteins (M. G. Hardinge and F. J. Stare, *Am. J. Clin. Nutrition* **2,** *83* (*1954*); *Nutrition Reviews* **13,** *138* (*1955*)). This, though moderately effective experimentally, would be unsatisfactory, inasmuch as it would disrupt the nutrient balance of the diet of our population to a considerable degree.

Another method for decreasing cholesterol would depend upon a supplement which could be taken *in addition* to a conventional diet. A few of the many attempts at achieving such a form of therapy are: (1) massive doses of nicotinic acid (R. Altschul and A. Hoffer, *Brit. Med. J.* **2,** *713* (*1958*), *Nutrition Reviews* **17,** *78* (*1959*)); (2) feeding of "Seed Fats" containing highly unsaturated fatty acids (H. Gordon *et al.*, *Nature* **180,** *923* (*1957*), *Nutrition Reviews* **16,** *131* (*1958*)); (3) inhibition of cholesterol synthesis by giving sodium alpha phenylbutyric acid, (J. Cottet *et al.*, *Bull. Acad. Nat. Med., Paris* **25–27,** *441* (*1953*), *Nutrition Reviews* **15,** *309* (*1957*)); (4) inhibition of absorption of fats and lipids by administration of beta sitosterol (M. Best *et al.*, *Am. J. Med.* **19,** *61* (*1955*), *Nutrition Reviews* **14,** *39* (*1956*)); and (5) acceleration of metabolic catabolism of cholesterol by administration of thyroid hormone or L-triiodothyronine (B. Strisower *et al.*, *Lancet* **I,** *272*, *120* (*1957*), *J. Clin. Endocrinol. Metab.* **19,** *117* (*1959*)).

Obviously none of these methods has proved to be of outstanding value, though each has its advocates. Perhaps we are attempting to treat a "laboratory value", for the causes of hypercholesterolemia are many. Consider the possible differences in the mechanisms which increase the cholesterol in each of the following: diabetes mellitus, familial hypercholesterolemia, myxedema, nephrosis, or gluttony. If we could demonstrate a metabolic defect common to all these conditions, the task would be simplified, but such is not the case.

And now another agent has been rediscovered to have a cholesterol lowering effect. Vanadium was found to inhibit synthesis of cholesterol in vitro (G. L. Curran, *J. Biol. Chem.* **210,** *765* (*1954*)). He found that vanadium would inhibit deposition of cholesterol in the aorta of rabbits, (*J. Exp. Med.* **103,** *49* (*1956*)). He postulated that the inhibition of synthesis was accomplished at two sites; conversion of mevalonic acid to hydroxymethylglutaric acid, and conversion of this compound to beta methylcrotonic acid.

This prompted Charles E. Lewis to study the effects of industrial exposure to vanadium upon the blood cholesterol level of workers (*Arch. Indust. Health* **19,** *419*,

497 (1959)). Vanadium poisoning, while seldom or never fatal, has been recognized as a source of annoying irritation of the mucous membranes of the respiratory tract. This condition usually occurs in association with the cleaning of boilers or heat exchangers fired with fuel oils containing a large amount of vanadium. Accordingly, Lewis selected 32 men who were exposed to vanadium fumes or dust, and a similar group of control subjects from the same localities (Colorado and Ohio). To lessen the effects of age upon cholesterol values, the men chosen were over the age of 40. All were free of apparent disease, and their jobs provided similar physical activity.

Because vanadium is excreted rapidly from the kidneys, the author employed determinations of 24-hour urinary excretion of this element to estimate degree of exposure. He eliminated eight men whose jobs did not expose them to excessive amounts of vanadium. In the final analysis, the exposed men excreted four times the quantity of vanadium as did their fellow controls.

Next he compared the concentration of cholesterol in their serum, and found a mean difference of 23 mg. per cent (228 compared with 205) which proved to be significant statistically. Other tests such as the electrocardiogram and the hematocrit disclosed no difference between the two groups.

Vanadium would appear to provide another clinical tool with which to study the synthesis and metabolism of cholesterol. As yet, it has not been employed as a therapeutic agent in patients with hypercholesterolemia.

CONGENITAL MALFORMATIONS FOLLOWING AMINOPTERIN INGESTION BY THE MOTHER

It is now well established that congenital malformations can be induced in mammals by maternal dietary deficiencies (*Nutrition Reviews* **13,** *289 (1955)*; **15,** *225 (1957)*). It has been generally accepted by workers in the field of maternal nutrition that congenital anomalies due to spontaneously occurring nutritional deficiency have not been recognized in the human being. Even in animal experiments, powerful vitamin antagonists have been used to control the degree and timing of maternal deficiency in order to produce consistent malformations in the offspring.

The administration of aminopterin, a powerful folacin antagonist, has been shown to produce fetal death in rodents. It has also been used to induce therapeutic abortions in women with tuberculosis or cancer (J. B. Thiersch, *Am. J. Obstet. Gynec.* **63,** *1298 (1952)*). In three out of 12 cases given oral doses of 6 to 12 mg. of aminopterin, immediate death did not occur and congenital malformations were noted in the fetuses.

J. Warkany, P. H. Beaudry and S. Hornstein (*J. Dis. Child.* **97,** *274 (1959)*) have reported an instance of congenital anomalies following the ingestion of aminopterin by the mother during early pregnancy. This 27-year-old mother of six living and normal children had purchased aminopterin tablets from her druggist and had taken 1.0 mg. daily for 12 days, beginning at the fifty-sixth day of gestation. She then showed symptoms compatible with aminopterin poisoning and was treated with folacin and citrovorum factor, which relieved these symptoms. The pregnancy was subsequently uneventful, and she had a spontaneous delivery approximately two weeks after the calculated date.

The infant's birth weight was 1,280 g. and length was proportionate. The head was large and soft with absence of ossification of the parietal bones, while the external ears were low set and seemed retained near the midline. The lower jaw was small. Moreover, there were malformations of the

helix of both ears and a posterior palate cleft. Despite the small body size, however, there were no other obvious anomalies.

Routine hematologic findings were within normal limits. Upon X-ray examination, the skull showed only ossification of the occipital bone and of relatively small portions of the frontal bones. There was delayed ossification of the pubis and ischium and bilateral dislocation of the hips, as well as clubbing and synostosis of the hands and feet, and short radii.

This infant died at the age of 29 hours. Autopsy findings showed that the brain, liver and kidneys appeared more mature than that of a normal newborn and that the ovaries, adrenals and cerebellar cortex showed a degree of immaturity in keeping with the small body weight. Except for some interstitial pneumonia, the microscopic findings were not remarkable.

An extensive family history failed to reveal any congenital anomalies in either the maternal or paternal side of the family.

Warkany, Beaudry and Hornstein have discussed the evidence associating the congenital anomalies in this infant with aminopterin ingestion by the mother. Previous animal and human experience suggested that aminopterin in similar doses could produce death or anomalies of the fetus when given during the first six weeks of gestation. Could the anomalies seen in this infant be attributed to the application of a teratogenic agent during the tenth to the twelfth week of the gestation?

In comparing the skull of this child and that of a nine week embryo (eleventh week of gestation), it was noted that the stage of ossification was similar to that seen in the reported case. Available evidence would suggest that final closure of the posterior palate may not take place before the fifty-sixth day of gestation, the assumed time of the beginning of aminopterin administration. The possibility that the deformity of the ear could have occurred at the same gestational age could not be excluded.

It was impossible to exclude a causal connection between aminopterin ingestion and the congenital anomalies on the basis of the gestational age at which they probably occurred. Yet the establishment of a direct cause and effect relationship was not possible in this case. The absence of a family history for similar defects would tend to rule out, but not definitely exclude, abnormal genetic factors. Consequently these authors felt that it was reasonable to assume that the anomalies of the child could be attributed to unsuccessful abortion attempts with aminopterin.

This case demonstrates that in the human being a nutritional insult to the fetus of appropriate intensity and timing may be followed by the development of congenital anomalies. The observations in this case fit in so well with those in experimental animals that there is little reason to question the biological significance of these findings. Furthermore, these observations reinforce the prevailing opinion that dietary deficiencies suitable for the production of congenital anomalies in the human being are not likely to occur spontaneously. The potential dangers surrounding the use of potent anti-metabolites could not be presented more dramatically.

SERUM PROTEIN LIPID AND LIPOPROTEIN FRACTIONS IN NORMAL HUMAN PREGNANCY

Using moving boundary electrophoresis, it has been reported earlier that significant alterations are found in almost all protein fractions of serum obtained from pregnant women when compared to nonpregnant women. The changes progress to a maximum

late in the third trimester and return to normal after delivery. In general, the albumin and gamma globulin values decrease while the alpha globulin values increase. There is also an increased level of fibrinogen in the plasma obtained from pregnant women. It has also been noted that there are increases in the serum cholesterol, cholesterol esters, phospholipids and neutral fat values in pregnancy with maximum levels being attained late in the third trimester.

Recently, E. K. Smith, R. R. de Alvalrez and J. Forsander (*Am. J. Obstet. Gynec.* **77,** *326 (1959)*) undertook to study the pattern of the serum protein and lipids during normal pregnancy. They used paper electrophoresis, which required only small samples of serum, and determined distribution of protein and lipoprotein by using the proper staining techniques. Initially ten women, observed at monthly intervals during the course of pregnancy and puerperium, formed the group to be studied. However, during the course of the investigation, one of the laboratory technicians who volunteered to serve as a nonpregnant control, became pregnant. Blood samples for analysis were obtained in this individual in the fifth week of pregnancy before the diagnosis of the condition was suspected. She was followed at approximately four week intervals throughout the pregnancy, delivery and at more frequent periods during the early and late postpartum periods. The data concerning protein and lipoprotein fractions obtained from the other patients in the group were of a similar nature to those from this individual, and were presented in full since they covered a time span from the nonpregnant period through the delivery and the late postpartum period.

It was noted that the serum albumin, expressed as per cent of the total serum protein, decreased progressively as the pregnancy advanced. At the fifth week of pregnancy albumin comprised 55.3 per cent of the total protein, while nine hours postpartum it had fallen to 42.1 per cent with the lowest value obtained four days postpartum (34.9 per cent).

It is of interest that the initial value of 55 per cent was not reached, even at six months postpartum when the albumin comprised 50.3 per cent of the total serum protein. The authors note, however, that the value of 55 per cent at the fifth week of pregnancy was high as compared to their group of normal nonpregnant women, whose serum albumin averaged 51.5 per cent of the total protein. Along with the decrease in serum albumin, there was an increase in both the alpha-1 and alpha-2 globulins. The alpha-1 globulin started at 4.7 per cent of the total proteins and increased up to 10 per cent on the fourth day post partum. The alpha-2 globulin started at original values of 9.7 per cent of the total protein and reached a maximum of 17 per cent four days post partum. It is of interest that while the alpha-1 globulins were high on the fourth day post partum, they practically disappeared 45 days later. Except for an increase during the later post partum period, the level of gamma globulin expressed as per cent of the total serum protein did not change during the entire study.

Some of the earlier investigators have reported a decrease in gamma globulin fractions occurring in pregnancy. The reduction in gamma globulin and albumin fractions as well as total plasma protein of nonpregnant patients is usually observed when patients have been on a diet deficient in protein. However, the history, personal observations and evaluations of the dietary intake of the patient under study indicated that she consumed liberal amounts of protein during the pregnancy and was in excellent nutritional status. Thus, the reduction in albumin which she showed was probably more directly attributable to the pregnancy, perhaps related to increased blood volume and so forth, than to the protein deficiency per se.

It was noted that the cholesterol, both free and ester, and the phospholipid content of the serum increased as pregnancy progressed. All three of these lipid fractions reached their maximum values at term and their return to a normal nonpregnant level was slow, with all showing some elevation above the initial value six months after delivery.

Since in calculation, the sum of percentages of alpha and beta lipoproteins equals 100, the percentage of the beta will always be 100 minus the concentration of the alpha. Since there was a sharp decrease in the alpha lipoproteins during the third trimester (from 35 per cent in the fifth week to 22.5 per cent in the third trimester), there was a correspondingly sharp increase in the beta lipoproteins. This simultaneous increase in beta lipoproteins appears to correlate well with the increased cholesterol and lipid phosphorus value which occurred in the patient during pregnancy.

J. T. Anderson and A. Keys (*Clin. Chemistry* **2,** *145 (1956)*) reported that the beta lipoprotein cholesterol, that is the lipoprotein separated by paper electrophoresis, represents 81 per cent of the total cholesterol in serum. Hence one would expect that an increase in the beta lipoprotein would occur in pregnancy as the cholesterol and cholesterol esters increase. However, as should be noted in the present work, the lipoprotein distribution returned to normal a bit more rapidly than did the concentration of cholesterol and lipid phosphorus.

Since there is a slow return to the original values for the serum albumin, the alpha-2 globulin, the cholesterol and the lipid phosphorus levels, these investigators (and some of the previous ones), suggested that the requirements for physiologic and metabolic readjustment with respect to protein and lipid metabolism is finally attained after a rather long period. They suggest that this time interval may be longer than six weeks post partum.

Summary

Using paper electrophoresis, serum determinations for total serum proteins, protein fractions and lipoprotein distribution were carried out. Also, serum cholesterol, cholesterol esters and lipid phosphorus were studied in a normal primigravida, beginning at five weeks of pregnancy and continuing at frequent intervals through delivery and up to six months post partum. As pregnancy progressed, a decrease in the serum albumin and the alpha globulin was noted while the beta globulin increased during the same period. The serum cholesterol, lipid phosphorus, beta-lipoprotein, as well as total lipids, also increased progressively to a maximum early in the puerperium. All changes were reversed following delivery, although some took at least six months to return to their initial value.

SERUM TRANSAMINASE LEVELS IN LAMBS WITH MUSCULAR DYSTROPHY

A naturally occurring type of muscular dystrophy occurs in lambs and calves which are raised in certain areas of Montana and some other states. There appears to be no association between this disorder and low levels of tocopherol in the ration, nor with low blood, colostrum or milk vitamin E levels. The disease appears to occur in lambs and calves born of mothers who have been fed hay which has either been grown or cured in affected areas. At present, it is not known whether this disease is caused by nutritional deficiency in the feed or may be produced by the presence of some toxic agent.

It is known that the enzyme glutamic-oxalacetic transaminase occurs in high levels in myocardium, liver and skeletal muscle, and destruction of the cellular integrity of these tissues leads to an increased level of

the transaminase in the blood. This increased blood enzyme activity has been used diagnostically, particularly in the detection of myocardial infarction. Also, increases in serum transaminase activity were noted in patients undergoing extensive surgical operations (*Nutrition Reviews* **16,** *204 (1958)*), and were believed to be due to liberating the enzyme from necrosing muscle cells.

C. Blincoe and W. P. Dye (*J. Animal Sci.* **17,** *224 (1958)*) have reported increased serum glutamic-oxalacetic transaminase activity in lambs raised in Nevada which had naturally occurring muscular dystrophy.

K. F. Swingle, S. Young and H. C. Dang (*Am. J. Vet. Res.* **74,** *75 (1959)*) have carried out studies to further investigate the relationship of serum glutamic-oxalacetic transaminase in lambs who were developing nutritional muscular dystrophy. A total of 418 lambs from the same flock were studied. Blood samples were taken routinely from each of the surviving animals when seven and 14 days old. Three-hundred and eighty samples were taken on the seventh day and 374 samples on the fourteenth day. Blood samples were also taken from lambs three to 47 days old in an attempt to correlate the serum glutamic-oxalacetic transaminase level with the observed signs and symptoms of the illness.

Of the lambs studied, 77 either developed clinical signs of muscular dystrophy or had lesions comparable with the disease at autopsy. On the average, the first signs of the disease appeared about 23 days following birth, although close daily observation was not possible under the conditions of the study. In the seven-day-old lambs which had serum transaminase levels between 50 and 99 units, the incidence of muscular dystrophy was 8.3 per cent. As the serum transaminase value increased between 100 and 149 units, the incidence of muscular dystrophy rose to 16 per cent.

In animals having serum transaminase values between 150 and 199, the incidence of muscular dystrophy rose to 23.8 per cent, and as transaminase levels rose, the incidence of muscular dystrophy also increased until 71 per cent of the animals which had serum transaminase values between 400 and 950 showed muscular dystrophy. It should be noted that in the last group there were only seven animals out of a total of 380 studied.

A similar trend was observed when the serum transaminase levels were determined on the 14-day-old lambs, although the incidence of muscular dystrophy at each of the levels was a little less than when the animals were seven days old. It should be noted that few specific diseases, other than muscular dystrophy, occurred in the flock. Bronchial pneumonia was the exception and occurred in many lambs. However, in none of the animals with pneumonia was there an increased serum transaminase activity noted at any stage of the disease.

These studies indicate that the serum glutamic-oxalacetate transaminase might be used as a sensitive test for the early indication of muscular damage in lambs developing muscular dystrophy. Apparently, as the muscle is damaged, there is a leakage of the enzyme from the muscle cell which accounts for the increased blood levels.

PENICILLIN IN GERM-FREE CHICKS

Previous studies have indicated that antibiotics have little influence on the growth of chicks and turkeys raised under germ-free conditions (T. D. Luckey, H. A. Gordon, M. Wagner and J. A. Reyniers, *Antibiotics and Chemotherapy* **6,** *36 (1956)*; M. Forbes, W. C. Supplee and G. F. Combs, *Proc. Soc. Exp. Biol. Med.* **99,** *110 (1958)*). The consensus seems to be that antibiotics exert their influence only by action on the intestinal microflora.

M. Forbes and J. T. Park (*J. Nutrition*

67, *69* (*1959*)) have issued a report, based on large numbers (350) of germ-free chicks and experimental "infection", which strongly confirms this contention. The method used for rearing germ-free chicks was patterned after that of J. A. Reyniers *et al.* (*Lobund Institute Report* **2,** *3* (*1949*)). The germ-free and conventional chicks were weighed and subdivided into two groups of chicks of approximately equal average weight. One group served as a control while the other received the diet supplemented with penicillin.

One casein-starch diet (C-1) with two modifications (C-1R) and (C-7), and one soybean-corn meal diet (C-8) were used. The main difference between diets C-1 and C-7 is that in the C-1 diet most of the inorganic phosphate is provided as potassium mono-hydrogen phosphate, while in the C-7 diet it is in the form of calcium phosphate. The C-1R diet differed from the C-1 in containing extra thiamine (five to ten times the requirement). Under germ-free conditions, the chickens were given canned sterilized water. Penicillin (potassium or procaine) was administered as powder mixed with starch. Samples of food, water and fecal material from each chick were tested for sterility.

Growth of the germ-free chicks on the C-7 diet occasionally appeared to be slower than on diet C-1. The authors suggest that the reason for slower growth may have been the unavailability of the calcium and phosphate fed as calcium phosphate. Diet C-1R, containing adequate thiamine, was found to give a significantly better growth than diet C-7, and adequate growth was also obtained with diet C-8, a soybean-corn meal diet with a high thiamine supplement.

On the "natural" diet (C-8) growth of the germ-free chicks was 18 to 25 per cent better than that of chicks from the same batch reared in the animal room. Again, on the C-1R diet, the germ-free chicks showed increased weight gain compared to the conventional chicks. The germ-free chicks which grew at a faster rate consumed about the same amounts of food per gram of body weight as the contaminated chicks. Thus, average food efficiency was the same for the germ-free birds as for conventional chicks, but for some reason the germ-free birds ate more. Food efficiency was 2.7 on the casein-starch diet and 4.4 on the soybean-cornmeal diet.

In repeated experiments extending over a period from January 1956 to January 1958, potassium penicillin (25 mg. per kg.) or procaine penicillin (45 mg. per kg.) failed to elicit growth responses in the germ-free or conventional chicks. The conventional chicks however, had not been infected with an extensive chicken flora, since no chicks had been raised in the animal room prior to these experiments. Thus presumably the animal room flora were not the types encountered in the usual chick laboratory.

In order to test this hypothesis, lyophilized intestinal contents were obtained from chicks reared in the animal rooms of the National Institute for Research in Dairying, Shinfield, England, where a growth response to penicillin was regularly obtained. One or two grams of this material was mixed with 2 kg. of C-1R diet, after which the penicillin supplement was added to half the ration. In this experiment and in all subsequent experiments, a growth response to dietary penicillin was observed. After three successive experiments, a growth response to penicillin supplements was seen even when the lyophilized intestinal material was no longer mixed with the diet. Comparison of the weights of the chicks in these experiments with those of the chicks from experiments performed immediately before contamination of the animal room, indicated that the growth of the contaminated chicks was depressed and that penicillin reversed this growth inhibition.

Comparison of the growth response to penicillin of germ-free chicks with that of chicks raised in the contaminated animal room indicated that penicillin mixed with the diet stimulates the growth of the conventional but not that of the germ-free

birds. Moreover, penicillin did not accelerate the growth rate of the conventional chicks to a level equal to that of the germ-free chicks.

In summary, growth of the germ-free birds was greatest, due to greater consumption of food. Growth of conventional but not specifically "infected" birds, was the same as that of "infected" penicillin treated chicks. The "infected" and non-penicillin treated chicks grew at the slowest rates presumably because of growth-inhibiting intestinal flora which could have been overcome by penicillin had it been given.

ALGAE AS A SOURCE OF HUMAN FOOD

During recent years great concern has been expressed about the shortage of food which probably will develop as the populations of the world increase. Extrapolating to the future, people will begin to starve during the foreseeable future unless reproduction is limited sharply or sources of food are increased at an unprecedented rate. While improved methods of agriculture undoubtedly will increase the available supply of food, the extent to which this will be possible is limited by the area of tillable land.

More recent interest in other sources of food production has been heightened by the prospects of interplanetary travel. The problem of supplying food for inhabitants of space stations or for the survival of pilgrims to the barren globes of the universe cannot be solved unless some means are devised to provide food with a minimum of transportation.

Since 1949, studies have been conducted on algae, the microscopic green plants which thrive in many waters of the earth (*Nutrition Reviews* **9,** *347, (1951)*). It was disclosed that cultures of *Chlorella* could produce food at a rapid rate when given optimal conditions of culture medium, carbon dioxide and sunlight. These are precisely the conditions that could be created in a space station; *Chlorella* could utilize the carbon dioxide exhaled by human inhabitants of such an enclosure, and, by producing food through photosynthesis, oxygen could be restored to the artificial atmosphere.

The efficiency of this tiny plant is indicated by a measure of food production called "the degree of reduction" which is based upon the ability of photosynthesis to perform an endothermic reduction of CO_2 and H_2O, liberating O_2 and storing energy. *Chlorella* rates higher than corn leaves, sunflower, or sugar maple wood, and by analysis it contains admirable quantities of protein (up to 58 per cent) as well as carbohydrates and fat. It has the ability to undergo constant mutation in response to environmental manipulations, a fact which permitted the development of strains capable of producing large quantities of protein. Thus, it appears to offer promise as a rich source of energy and nutrition to a future population.

Other investigators have utilized the effects of environmental changes upon growth characteristics of *Chlorella* to study the possibility of producing oils (*Nutrition Reviews* **11,** *77 (1953)*). By appropriate methods it has been possible to increase the lipid content of *Chlorella* to 86 per cent of the dry weight. Thus it seems reasonable that much of the world's supply of fats and oils could be produced in this fashion if the necessity arose.

Nutritional analyses of *Chlorella* have shown it to contain an abundance of vitamin A and ascorbic acid as well as adequate concentrations of the essential amino acids, except methionine.

By applying mathematical extrapolation from laboratory yields of *Chlorella*, it has been predicted that one acre of culture medium could produce about 40 tons of

dried high-protein food per year. However, the problems of sterility, quality control and processing would be costly and difficult.

This extravagance, great though it may be, seems less formidable when one considers that vegetable foods (grains, hay and forage) are grown primarily for animal feed. The efficiency of conversion to meat is less than 30 per cent (more often 5 to 10 per cent), and thus the relative cost of producing *Chlorella* for human consumption would be reduced to one third or less of its apparent expense (*Nutrition Reviews* **12,** *111 (1954)*).

Since 1954, a group of Japanese scientists have been conducting extensive studies on *Chlorella* as a source of food (*Nutrition Reviews* **13,** *13 (1955)*). They demonstrated that palatable dried powders could be added to such foods as bread, ice cream, noodles, and similar staples, to increase their content of protein, vitamin A and ascorbic acid. They reported that these foods had an agreeable taste and a greenish color which was not unpleasant. As might be expected the Japanese are vitally interested in the potential of algae as a source of food supply since their population far exceeds the capacity of their farm land to support its needs.

In the *Annual Report of the National Institute of Nutrition* (The National Institute of Nutrition, Toyamacho, Tokyo, Japan (1958)), two groups of scientists published reports on studies of algae. The first group (E. Tamura, *et al.*) described (in 13 papers) some chemical attributes of *Chlorella* and *Scenedesmus*. In agreement with previous reports, they found these algae to be rich in protein, to contain adequate amounts of the essential amino acids except methionine, and to provide an abundance of vitamin A. However, in contrast to earlier reports, they described adverse factors; the dried powder was accepted poorly due to its "odor, flavor and color", and it was digested incompletely. Decolorizing the dried algae with methyl alcohol not only reduced the odor and color, but also improved its digestibility. Weanling rats fed dried *Chlorella* (or *Scenedesmus*) grew poorly when compared with similar rats fed casein. Decolorization of the algae improved the growth of the rats but the product continued to be inferior to casein. The addition of methionine provided adequate nutrition to support optimal growth.

Electron - microscopic examination of fecal specimens from rats and from men fed these dried algae disclosed that the cellulose membrane remained intact when either the dried or decolorized forms were consumed, but absorption was better from the latter.

The second group reporting in the same publication (H. Hayami and K. Shino) described the effects of feeding a diet containing 19 per cent decolorized *Chlorella* to young white rats. The growth of these animals was compared with that of a control group fed casein, and another group fed dried *Chlorella*. Growth responses were: decolorized *Chlorella* 84.8 g., casein 95.5 g., and dried *Chlorella* 66.6 g. Accordingly they concluded that the nutritive value of *Chlorella* was improved by the decolorizing process, but was not equal to that of casein.

Next, these same workers investigated the rate of absorption of decolorized *Chlorella* in men. They found that much of the objectionable taste and odor of *Chlorella* was removed by decolorization, but "the remaining smell and taste of the decolorized *Chlorella* were intolerable and impaired the appetite." The five men were fed a basal diet containing 2300 calories and 60 g. of protein per day. Three days later, in addition to their basal diet, they were given 30 g. of decolorized *Chlorella* (21 g. of protein). In the final three day period they were fed the basal diet and 100 g. of beef. The "true digestibility" of the three dietary

regimens was calculated to be: basal diet 82.9 per cent, decolorized *Chlorella* 83.0 per cent, and beef 87.7 per cent. It was apparent that the addition of this amount of *Chlorella* to the basal diet was of no detectable benefit.

From these and previous studies, we can conclude that algae do have nutritive value which could be applied to human nutrition. The obvious objections to such application are the cost of production and processing, the relative deficiency of certain vital nutrients such as methionine, the incomplete digestibility, and poor acceptability due to odor, flavor and color. Certainly, if the need for additional sources of food continues (as it undoubtedly will) the value of this form of investigation should be great.

BIOTIN AND UNSATURATED FATTY ACIDS AS BACTERIAL GROWTH FACTORS

Several theories have been advanced to explain the ability of certain unsaturated fatty acids to replace biotin as essential growth factors for certain gram-positive bacteria (C. Nieman, *Bacteriological Reviews* **18,** *147* (*1954*); *Nutrition Reviews* **16,** *116* (*1958*)). However, in the light of present knowledge that biotin functions in the synthesis of the long chain fatty acids in both animals (D. M. Gibson, E. B. Titchener and S. J. Wakil, *J. Am. Chem. Soc.* **80,** *2908* (*1958*)) and plants (E. B. Kurtz, Jr. and A. Miramon, *Arch. Biochem. Biophys.* **77,** *514* (*1958*)) it appears probable that its function in this case lies in the biosynthesis of the necessary unsaturated acids as proposed by W. L. Williams, H. P. Broquist and E. E. Snell, *J. Biol. Chem.* **170,** *619* (*1947*)). If this hypothesis is true, the fatty acids thus supplied must be incorporated into the cells where they perform some useful function or are transferred into substances which do.

In order to investigate this possibility, W. M. O'Leary (*J. Bacteriol.* **77,** *367* (*1959*)) grew cultures of *Lactobacillus arabinosus* strain 17-5, ATCC 8014 in media adequate in all respects except that biotin was replaced by tween 40 solutions of carboxy-labeled oleic (9-octadecenoic) or cis-vaccenic (11-octadecenoic) acids, which are the major fatty acids with growth promoting activity for these organisms. After incubation for the appropriate time, cells were harvested and lyophylized. The fats were hydrolyzed and the fatty acids extracted. They were separated and analyzed by rubber column chromatography after oxidation of unsaturated acids to the dihydroxy acids. When cis-vaccenic acid was used in the medium the isolated fatty acids contained 22.5 per cent of dihydroxy acids, which were shown by mixed melting point to be largely the 11,12-dihydroxy acid from cis-vaccenic and 39.5 per cent of C_{19} acids, which were shown by microbiological assay to be almost entirely lactobacillic (cis-11,12-methyleneoctadecanoic) acid, an important constituent of the lipids of lactobacilli.

The total number of moles of these two acids was equal to 95 per cent of the amount of cis-vaccenic acid which had disappeared from the medium. Moreover, the activities of these acids were approximately equal and amounted to about 95 per cent of the activity of the acid supplied to the medium. Of the other acids separated from the bacterial lipids, only palmitic showed any activity (about 2 per cent of that of vaccenic acid).

It thus appears that the vaccenic acid taken up by the cells was transformed directly into lactobacillic acid without dilution and with little other transformation of starting material or product.

When oleic-1-C^{14} acid was supplied to the medium almost identical results were obtained. Again, the dihydroxy acid formed (27 per cent of the total) was derived from cis-vaccenic acid and the C_{19} acids (33 per cent of the total) were almost exclusively

lactobacillic acid. However, only 87 per cent of the oleic acid which disappeared from the medium was accounted for in the two acids considered above and the activity of these two acids was only 85 per cent of that of the oleic acid.

It appears from this study that oleic acid is isomerized by these organisms to cis-vaccenic, which is transformed as in the other experiment into lactobacillic acid. The 15 per cent loss may be the result of an incomplete isomerization of oleic acid and a resultant loss by oxidation or other mechanism.

These studies have indicated that the fatty acids capable of sparing biotin in these organisms are actually incorporated into the cells of the organisms and are transformed into lactobacillic acid, which is a typical and vital fatty acid constituent of the lipids of lactobacilli. Evidence has thus been provided that a major function of biotin in this case as in animals and higher plants is in the biosynthesis of these fatty acids.

METABOLISM OF UNESTERIFIED FATTY ACIDS

One of the recent significant developments in the field of lipid metabolism is the recognition that unesterified fatty acids in the blood play a biologically important role (*Nutrition Reviews* **16,** *102, 135 (1958)*). V. P. Dole and his associates and R. S. Gordon, Jr. and his associates verified the earlier finding of Szent-Gyorgy and Tominaga (*Biochem. Zeit.* **146,** *226 (1924)*) that blood normally contains a small amount of unesterified fatty acids.

The more recent workers have gone on to demonstrate that these fatty acids contribute significantly to the total metabolism of both man and animals. The level of these fatty acids in the blood fluctuates in an inverse relation to the level of carbohydrate metabolism. Those factors which enhance the rate of carbohydrate metabolism, reduce the level of unesterified fatty acids in the blood.

The previous work of Gordon and associates suggested very strongly that the adipose tissue is a source of the unesterified fatty acids (*Nutrition Reviews* **16,** *135 (1958)*). More recent work by these investigators provides additional evidence that the adipose tissue is a source of the blood unesterified fatty acids. R. S. Gordon, Jr. and A. Cherkes (*Proc. Soc. Exp. Biol. Med.* **97,** *150 (1958)*) studied the effect of insulin on the *in vitro* liberation of unesterified fatty acids from adipose tissue. They used male rats weighing between 150 g. and 200 g. The animals were fasted for 18 hours when the epididymal fat depots were removed. This tissue occurs in thin leaves and does not require slicing for *in vitro* studies. The tissue was rinsed in a 1 per cent solution of bovine serum albumin in saline and then placed in Erlenmeyer flasks containing a solution of bovine serum albumin in Krebs-Ringer phosphate buffer.

Their studies indicated that the nutritional state of the animal had a pronounced effect upon the *in vitro* liberation of unesterified fatty acids by the epididymal fat pad. The rate of production was markedly reduced when the rats were given a 10 per cent glucose solution for the 18 hours prior to sacrifice when compared with the rats that had been starved (0.2 versus 6.4 μM unesterified fatty acids per gram of tissue per hour, respectively).

In similar experiments with rats that had been starved for 18 hours, the adipose tissue that was incubated with insulin produced less unesterified fatty acids than the contralateral control tissue (-1.0 versus $+1.6$ μM per gram of tissue per hour, respectively).

These workers stated that the liberation of unesterified fatty acids from the adipose tissue in experiments *in vitro* supported their earlier conclusion. Their thesis is that those

factors that enhance carbohydrate utilization (*e.g.* insulin) reduce the liberation of unesterified fatty acids, while those that increase the requirement for calories (*e.g.* starvation) increase it.

The arterio-venous differences in their fasting subjects (*Nutrition Reviews* **16,** *102* (*1958*)) indicated that the source of the circulating unesterified fatty acid was adipose tissue. Gordon and Cherkes (*loc. cit.*) calculated that if the adipose tissue were the primary source of the unesterified fatty acids, and if that tissue were to account for a significant fraction of the total metabolism of the rat, the adipose tissue should liberate approximately 100 micromoles of unesterified fatty acids per gram of tissue per hour. This rate is almost 20 times greater than that seen in any of their fasting rats (6.4 μM). They suggest that the low rate of unesterified fatty acid production in the *in vitro* experiments stems from the poor circulation, and that when the blood supply is intact the rate might be more nearly sufficient to account for the energy requirements. Although the amount of insulin incubated with the adipose tissue was at a concentration two orders of magnitude higher than that effective *in vivo*, they still felt their results suggested that insulin acted directly on the adipose tissue rather than some other tissue or organ.

Additional evidence suggesting that certain tissues liberate, while others utilize, unesterified fatty acids has been presented by P. S. Roheim and J. J. Spitzer (*Am. J. Physiol.* **195,** *288* (*1958*)). They used 16 normal dogs that had been fasted over night. In practically all cases there was a higher concentration of unesterified fatty acids in femoral vein blood than in femoral arterial blood. These differences ranged up to 0.49 μM per liter, with most of them close to 0.15. The investigators interpreted this finding as indicating the release of lipids by the adipose tissue in the legs. There were no differences in the arterial and venous blood insofar as triglycerides, total and free cholesterol, or phospholipids were concerned.

The liver was shown to take up significant amounts of unesterified fatty acids. For these experiments they used dogs with a cannula implanted in the portal vein and another in the hepatic vein. In 22 out of 27 samples, the portal vein contained more unesterified fatty acids than the hepatic vein. The difference between the two blood samples was approximately 0.1 μM per liter, suggesting that the liver removes some unesterified fatty acids from the blood. Again, there was no difference in the levels of triglycerides, total and free cholesterol, or phospholipids in the two samples of venous blood.

Consecutive blood samples taken from the same animal over periods as long as three and one-half hours showed considerable variation in the absolute levels of the unesterified fatty acids. This was true for the samples taken from all sites. These fluctuations were explained by assuming that the ratio of uptake and liberation of fatty acids by different tissues varied within short intervals of time.

The behavior of the unesterified fatty acids in the blood during the absorption and transport of lipids has been studied by a number of investigators. M. I. Grossman, L. Palm, G. N. Becker and H. C. Moeller (*Proc. Soc. Exp. Biol. Med.* **87,** *312* (*1954*)) found that the level of unesterified fatty acids increased in the blood from a level of 0.35 μM to 0.59 μM per liter three hours after 2 ml. of corn oil were given (intragastrically) to rats that had been fasted for 16 hours. Although the level of unesterified fatty acids increased in the blood of the rats given the corn oil, the level of all fatty acids increased after the corn oil feeding to such an extent that the unesterified fatty acids represented a smaller percentage of the total than in the fasting state (3.9 versus 6.8 per cent).

Similar studies have been made in dogs by Spitzer and H. I. Miller (*Proc. Soc. Exp. Biol. Med.* **92,** *124* (*1956*)). They fed dogs

40 ml. of cod liver oil and removed blood samples at regular intervals. The esterified fatty acids in the blood increased from a fasting level of 10 μM per liter to 20 during the four hours after the ingestion of the oil. The unesterified fatty acids remained low during the first three hours and then showed a transient increase between the third and fifth hours. The above observations suggested to the investigators that the unesterified fatty acid fraction is of minor importance for the absorption of exogenous lipids.

Spitzer and Miller (*loc. cit.*) studied the role of unesterified fatty acids during the transport of endogenous lipids. The lipemia was produced by fasting a dog three days. During this time the unesterified fatty acids increased from a level of about 0.35 μM per liter at the start of the fast to 1.0 μM during the second day of the fast, and remained there until food was given. Ingestion of food promptly reduced the level of unesterified fatty acids to 0.4 μM. During the period when the unesterified fatty acids were markedly elevated, the blood sugar decreased from a control level of 80 mg. per 100 ml. to about 65. Food promptly increased the blood sugar level. The esterified fatty acids followed a course somewhat similar to that of blood sugar.

Dogs that had been poisoned with carbon tetrachloride (10 ml. given subcutaneously on two consecutive days) showed a marked increase in the unesterified fatty acid level of the blood. This was associated with only a slight increase in the esterified fatty acid level. The marked increase in the liver fats following the carbon tetrachloride injection appeared to be the result of the transport of lipids from the depot by means of the unesterified fatty acids.

That unesterified fatty acids appear very rapidly in the blood during chylomicron metabolism has been shown by R. J. Havel and D. S. Fredrickson (*J. Clin. Invest.* **35,** *1025* (*1956*)). They used isotopically labelled chylomicrons which were secured from dogs in which a cannula had been placed in the thoracic lymph duct. Each dog was given 3.6 g. to 10.8 g. of fat in the form of cream by stomach tube. The cream contained sodium palmitate-1-C^{14}. The chylomicrons were separated from the lymph, washed, and resuspended in 0.5 M sodium chloride solution. The resulting emulsion was given by intravenous injection to two adult female dogs. These dogs had been fasted for 20 hours prior to the injection. During the injection, the dogs were maintained under light ether anesthesia. Physiologic saline was given by vein during the infusion of the chylomicrons and the collection of subsequent blood samples.

The chylomicrons disappeared exponentially from the blood with a half life of 15 to 24 minutes. The triglyceride and phospholipid fractions of the chylomicrons disappeared at the same rate. The specific activity of the plasma unesterified fatty acids rose during the clearing of the plasma and reached a level exceeding 50 per cent of the specific activity of the transfused triglyceride fatty acids. The work of Havel and Fredrickson (*loc. cit.*) showed that the half life of the esterified fatty acid fraction was about two minutes.

Havel and Fredrickson (*loc. cit.*) suggested that there is an intimate relationship between the removal of chylomicrons and the hydrolysis of their constituent triglycerides. The site where this hydrolysis of the triglycerides occurs is unknown. Regardless of the tissue responsible for the removal of the chylomicrons from the blood, part of the unesterified fatty acids released during this process is returned rapidly to the blood stream. Here the fatty acids combine with serum albumin and are transported as a protein-fatty acid complex.

The above work indicates that although the unesterified fatty acids form a small fraction of the total blood lipids, their rate

of turnover suggests that they play a very important role, especially in the transport of lipids from the adipose tissue to such organs as the liver. The liver appears able to remove these fatty acids from the blood. The rate of removal of the unesterified fatty acids by the liver appears to occur even when no other lipid fractions are being removed from the blood.

With the increasing activity in this field of lipid metabolism, it is likely that additional evidence will soon be forthcoming to delineate more clearly the exact role of unesterified fatty acids in metabolic reactions.

THE STRUCTURE OF VITAMIN K

Discussions of the vitamins K have usually included a consideration of three main structures: vitamin K_1 (2-methyl-3-phytyl-1,4-naphthoquinone, from higher plants), vitamin K_2 (2-methyl-3-difarnesyl-1,4-naphthoquinone, from microorganisms) and menadione (2-methyl-1,4-naphthoquinone, a synthetic product) (*Present Knowledge of Nutrition*, The Nutrition Foundation, Inc., New York, 1956). These structures present an unique and interesting problem in that all three are equally active as antihemmorrhagic agents on a molar basis; in other words, the synthetic product is most active on a weight basis.

This introduces the question of the structure actually active as a vitamin in the animal body. It has been considered possible that both natural vitamins could be converted to menadione in the animal body, or that menadione could be converted to a vitamin K by addition of an isoprenoid side chain. However, it seemed unlikely that a phytyl chain could be added in the animal body, and the exact nature of the added side chain, if any, had not been ascertained (*Nutrition Reviews* **14,** *211 (1956)*; **15,** *142 (1957)*).

In a series of experiments, C. Martius and his co-workers have concerned themselves with the structure and function of the vitamins K. Recently Martius and H. O. Esser, (*Biochem. Z.* **331,** *1 (1958)*), have described research leading to the unraveling of the structure of the animal vitamin. The 2-C^{14}-methyl-1,4-naphthoquinone was given to vitamin K-deficient chicks or rats orally or intraperitoneally (the latter to avoid the action of intestinal flora). Activity was found in all organs of the animals. Heart muscle was especially rich in the radioactive product, although the liver was the best source of adequate amounts of the derivative. Counter-current distribution of the active product (using methylglycol-heptane as solvent system) showed it to have become strongly lipophylic, unlike menadione, and to have a distribution similar to that of various vitamin K_2 analogues with side chains of 15, 20 and 25 carbons.

Attempts to separate the bis-(p-bromobenzoyl-) derivative of the radioactive product from those of the vitamin K_1 and the vitamin K_2 analogues were unsuccessful. However, it was found that these derivatives could be separated by counter-current distribution (using 50 per cent dimethylformamide-50 per cent methanol/heptane). The radioactive product exactly followed the distribution of the cis-trans and all-trans derivatives of vitamin K_2 (20 carbon side chain). Moreover, after hydrogenation, the distribution was exactly similar to that of dihydrovitamin K_1, showing that the side chain of the product formed in the animal body has 20 carbons and probably four double bonds (as was confirmed by quantitative hydrogenation). The structure of the animal vitamin K is thus elucidated as 2-methyl-3-(geranyl-geranyl)-1,4-napththoquinone. Geometrical configuration of the double bonds could not be ascertained since isomers of this type could not be separated.

Present knowledge of the biogenesis of squalene and cholesterol in the animal body has given a general pathway for formation of isoprenoids via the compound mevalonic acid (alpha, gamma-dihydroxy-beta methyl valeric acid). Martius and Esser reported experiments in which the side chain of the menadione derivative was synthesized from radioactive mevalonic acid by chicken liver homogenates, thus indicating its common origin with the other animal isoprenoids.

Assignment of a role of provitamin to menadione is difficult in view of its purely synthetic origin. However, the authors consider it possible that all dietary vitamins K are converted to menadione or a simple derivative before addition of the 20-carbon side chain.

Thus, it appears that a long search is approaching an end. The exact role of this compound in oxidative phosphorylation, however, is still to be worked out in final form.

PREGNANCY AND LIVER GLUCOSE-6-PHOSPHATASE ACTIVITY

The enzyme, glucose-6-phosphatase, has been found in high concentrations in the liver, while lower levels of activity are present in the kidney and traces are found in the intestine. This enzyme is responsible for removing the phosphate from the 6 carbon of glucose, and for the release of glucose from the liver into the peripheral and hepatic circulation. Its activity has been found to increase in the liver of alloxan-diabetic animals.

R. L. Burt (*Am. J. Obstet. Gynec.* **74,** *551 (1957)*) noted that pregnancy induced a diabetogenic effect on carbohydrate metabolism, which was characterized by impaired peripheral utilization of glucose and resistance to insulin. The question arose as to whether these changes might be caused by increased levels of glucocorticoids occurring during gestation. It has also been reported by R. G. Langdon and D. R. Weakley (*J. Biol. Chem.* **214,** *167 (1955)*) that the administration of cortisone or adrenal cortical extract to mice increased the level of liver glucose-6-phosphatase.

Recently, R. L. Burt and N. Julian (*Am. J. Obstet. Gynec.* **77,** *6 (1959)*) studied the glucose-6-phosphatase activity in the liver of albino rats who were sacrificed on the seventeenth day of gestation. (The normal gestation period of the rat varies from 21 to 30 days.) The determination of glucose-6-phosphatase was carried out in homogenates of the liver by measuring, under standard conditions, the increase in inorganic phosphate produced when this enzyme was acting on glucose-6-phosphate as a substrate. In studying the livers of 23 pregnant rats for glucose-6-phosphatase activity, they found that an average of 5.25 ± 1.23 (mean $\pm$ standard deviation) mg. of inorganic phosphorus was released per g. of liver. In 31 control animals, 6.98 ± 1.10 mg. of inorganic phosphorus was released per g. of liver.

The decrease in mean glucose-6-phosphatase activity in the liver of pregnant rats as compared to the control animals, was highly significant with a $P = <0.001$. This difference was also significant if the values were expressed per mg. of liver nitrogen rather than per g. of fresh liver. Thus, the decrease in the glucose-6-phosphatase levels of the liver in pregnant rats was an absolute value and not dependent upon the state of hydration.

With the decrease of glucose-6-phosphatase activity, one can theorize that this might be responsible for a decreased fasting blood sugar which has been reported to occur in human pregnancy, as well as for the increased liver glycogen reported by R. L. Burt and C. A. Kimel (*Am. J. Obstet. Gynec.* **74,** *295 (1957)*) to occur in the livers of pregnant animals.

The regulation of blood glucose and liver

glycogen is a complex phenomenon influenced by many factors. From the present study, it appears that the level of glucose-6-phophatase activity in the liver may be one of the factors responsible for the abnormal glucose metabolism in the pregnant animal. However, it has not been established whether these small but statistically significant changes in enzyme activity are sufficient to cause a physiological change, such as a reduction in blood sugar, since the tissues normally have a large reserve capacity of enzymes, and small changes in enzyme concentration would not necessarily result in gross physiological changes.

Also, while these investigators have shown that the addition of progesterone and estrogen did not increase the glucose-6-phosphatase activity in the liver of normal animals, the effect of other hormones (either direct or indirect) cannot be ruled out at present.

ADRENALS AND FAT MOBILIZATION

Many people interested in nutrition are inclined to focus their attention on nutrients and related compounds. Frequently tissues and organs apart from the gastrointestinal and circulatory systems are regarded as having only a minor role in the overall nutritional picture. A little deliberation soon indicates that practically all physiological reactions involving the digestion, absorption and assimilation of food are controlled by a variety of factors that are not directly related to the diet.

The hormones play an important role in many of these processes. Their regulation of nutritional reactions becomes apparent only when abnormalities develop in the endocrine system. The abnormalities may be either spontaneous as in certain diseases or induced by such techniques as surgical or chemical intervention.

F. Verzar and L. Laszt (*Biochem. Z.* **288,** *356 (1936); Nutrition Reviews* **13,** *207 (1955)*) secured evidence that the adrenals play an important role in the release of fat from the depots. This conclusion was based on the observation that the fatty liver which follows the administration of a dose of phosphorus did not occur in adrenalectomized rats. In the same way, the fatty liver which normally is seen when mice are starved for seven hours does not occur in adrenalectomized animals (L. Levin and R. K. Farber, *Recent Progr. Hormone Res.*, **7,** *399 (1952)*). Admittedly, these are but indirect bits of evidence implicating the adrenals in the mobilization of fat, since it is assumed that the fat appearing in the liver comes from the depots.

A number of investigators hoped to secure more direct evidence for the adrenal regulation of lipid levels in adipose tissue by studying the deposition of fat in the liver resulting from the administration of ethionine and the action of the adrenal hormones thereon. At the University of Chicago, I. G. Wool and M. S. Goldstein (*Am. J. Physiol.* **175,** *303 (1953)*) observed that when they injected female rats (130 to 200 g. body weight) with ethionine, a methionine antagonist, the animals developed livers with 11.8 per cent fat during the 24 hour fast following the ethionine injection. The injection was made after a 12-hour fast. The controls which were fasted without the ethionine had liver fat levels of 4.9 per cent.

When adrenalectomized animals were given ethionine, their liver fat levels were the same as those of the normal controls (5.8 per cent). The injection of a maintenance dose of either cortisone (0.3 mg.) or epinephrine (1 mg. per kg.) into the adrenalectomized rats produced no change in liver fat levels when ethionine was injected. When epinephrine and cortisone were injected together into the adrenalectomized

rats, the liver fat following ethionine was practically the same as that in the unoperated animals (10.1 vs. 11.8 per cent).

The above investigators concluded that "in the adrenalectomized animal a level of adrenal cortical steroids that by itself is ineffectual will permit the response to occur when combined with adrenomedullary hormone, epinephrine."

K. A. Brownell and F. A. Hartman (*Am. J. Physiol.* **196,** *593 (1959)*) extended the above work to the mouse, which according to them is more sensitive to lipid mobilization than the rat. The mouse differs from the rat, however, in that ethionine produces fatty livers in both males and females, whereas only the female rat responds to ethionine in this way.

Male albino mice (ten to 14 weeks of age) were injected with DL-ethionine (75 mg. per 100 g. body weight) in three equal doses two and one-half hours apart. The liver lipids 24 hours after the first injection were 16.9 per cent compared to 8.5 per cent in the uninjected control mice. Here, as with the rats, the mice received no food but water ad libitum after the ethionine injection.

The mice whose adrenal medulla had been removed 15 days earlier responded to the injection of ethionine with an increase in liver lipid levels (17.0 vs. 11.0 per cent in the injected and control demedullated mice respectively). When a single dose of 30 micrograms of epinephrine in oil was injected subcutaneously just prior to the ethionine, there was no significant increase in liver lipid levels. This observation suggests that epinephrine is not the essential adrenal hormone for the mobilization of fat from adipose tissue, at least in so far as mice are concerned.

The adrenalectomized mice were furnished with 1 per cent sodium chloride solution and food for the first 24 hours after the operation. During the second 24 hour period they were allowed to have water only. When these animals were treated with ethionine and given an injection of 60 micrograms of cortisone, the liver lipid levels were much less than those in the unoperated animals treated with ethionine (11.3 vs. 16.9 per cent respectively). The combination of cortisone and epinephrine had no greater effect than cortisone alone.

This suggests that either there is some factor(s) in the adrenal other than cortisone and epinephrine which is effective in the mobilization of fat, or the operative procedures changed the animal's response to ethionine.

The increase in liver fat levels following an injection of ethionine does not occur when the adrenals are removed. In the rat, a combination of epinephrine and cortisone appears able to replace the adrenals in so far as the above reaction is concerned. Neither the mixture of epinephrine and cortisone nor an extract of the adrenal gland appear able to produce as high levels of liver fat, following an injection of ethionine, in the adrenalectomized mice as in intact animals.

Additional indirect evidence for the action of the adrenal hormone in lipid mobilization comes from studies of blood lipid levels. When a single dose of epinephrine (0.75 mg. per kg. of body weight) in oil was injected into adult rabbits, the total lipids in the serum increased from a pre-injection level of 360 mg. to 951 mg. per 100 ml. 48 hours after the injection (A. Dury, *Circulation Research* **5,** *47 (1957)*). Neutral lipids accounted for the major part of the increase during the above period as shown by the change from 167 mg. per 100 ml. of serum prior to the injection to 618 mg. per 100 ml. 48 hours later. The epinephrine produced only slight increases in blood sugar levels, which suggested that the action of the hormone on the blood lipid levels was not mediated through its effect on carbohydrate metabolism.

The work of R. O. Scow, S. S. Chernick and B. A. Guarco (*Diabetes* **8,** *132 (1959)*) indicated that under certain conditions

cortisone can influence the level of blood lipids. When they injected as little as 0.25 mg. of cortisone per day into hypophysectomized-pancreatectomized rats, the blood lipids (non-phospholipids) increased from a level of 200 mg. to 300 mg. per 100 ml. Larger doses of cortisone (2 mg. per day) injected one hour after hypophysectomy raised the blood non-phospholipid levels to 600 mg. per 100 ml. 24 hours after the injection. The adrenalectomized-pancreatectomized rat responded in the same way. Cortisone, however, had no effect on the blood lipid levels of normal rats or in hypophysectomized rats with an intact pancreas.

Since the above rats received no food for 17 hours prior to the start of the study and for the duration of the experimental period, and since the blood lipid levels increased within a few hours of the injection, these investigators felt they had evidence for an action of cortisone on adipose tissue.

More direct evidence for the action of epinephrine on adipose tissue stems from the work of R. S. Gordon, Jr., and A. Cherkes (*Proc. Soc. Exp. Biol. Med.* **97,** *150 (1958)*). They found that the addition of epinephrine (0.01 μg. per ml.) to a bovine serum albumin, Krebs-Ringer phosphate buffer, increased the liberation of unesterified fatty acids from the epididymal fat pads of male rats (150 to 250 g. body weight). The control tissues produced 1.3 μM of unesterified fatty acid per gram of tissue per hour, while the tissue incubated with epinephrine produced 2.31 μM. A higher concentration of epinephrine increased the lipid liberated from the supplemented adipose tissue.

The above experiments strengthen the evidence that the adrenal hormones play an important role in the mobilization of fat, presumably from the adipose tissue. There is an accumulating body of evidence which implicates the adrenal cortex in this reaction. Certain corticoids appear to be involved in the deposition of fat in the liver following an injection of ethionine. Cortisone produces a pronounced increase in the blood lipid levels of hypophysectomized or adrenalectomized rats that become diabetic following the removal of the pancreas.

Epinephrine also is involved in some of these phenomena, since injections of this hormone in oil suspensions (which permit a longer period for its action) increases the levels of neutral fats in the blood. There is some evidence that epinephrine accelerates the mobilization of fat from adipose depots.

The interaction of these hormones and the mechanisms by which they influence and perhaps regulate the level of fat in the depots remain problems for future research.

MUCOPOLYSACCHARIDES AND ATHEROSCLEROSIS IN THE RABBIT

In atherosclerosis there are many factors which may influence the initiation and progression of the disease. Arterial injury, metabolic factors and diet may all play a role. Investigation must continue to evaluate the relative importance of these factors in the development of the disease process in different patients. In order to do this well we need more knowledge about the early histopathology of atherosclerosis.

I. Gore and R. Barr (*Lab. Invest.* **8,** *395 (1959)*) have discussed the evidence in favor of accumulation of lipid versus mucopolysaccharide as the initial lesion of atherosclerosis. Several lines of investigation have suggested the possibility that the deposition of mucopolysaccharides could play a primary role in the development of the disease.

J. F. Rinehart and L. D. Greenberg (*Arch. Path.* **51,** *12 (1951)*) reported experiments with pyridoxine deficient monkeys in which lesions developed resembling human atherosclerosis. Mucoid material

which stained metachromatically accumulated in the intimal ground substance. H. D. Moon (*Circulation* **16,** *263* (*1957*)) studied the coronary arteries in fetuses, infants and juveniles. He concluded that degeneration of the intimal elastic membrane accompanied by accumulation of mucopolysaccharide and fibroblastic proliferation was the early, non-lipid phase of atherosclerosis in infants and adults.

M. Dyrbye and J. E. Kirk (*J. Gerontology* **12,** *20* (*1957*)) in contrast to M. Faber (*Arch. Path.* **48,** *342* (*1949*)) showed a decrease with age in extracted, chemically determined sulfated polyuronide. In rabbits with cholesterol-induced atherosclerosis, G. C. McMillan and B. I. Weigensberg (*Circulation* **16,** *495* (*1957*)) were unable to demonstrate chemically or morphologically any alteration in acid mucopolysaccharide content.

In view of the importance of identifying the initial lesion of atherosclerosis Gore and Barr (*loc. cit.*) have observed the levels of serum cholesterol, mucoprotein, protein-bound hexose and hexosamine, as well as blood proteins including lipoprotein and glycoprotein in rabbits with cholesterol-induced atherosclerosis.

Weanling New Zealand female rabbits were fed rabbit pellets only or pellets coated with 400 mg. of cholesterol per day. Animals were killed after 22, 66, 94, 112 and 132 days. Although all animals were examined in a complete autopsy, the hearts and aortas received special attention. After formalin fixation, Sudan III was used to stain lipids deposited in the aorta. On the basis of the gross uptake of this stain (sudanophilia) representative samples were taken for microscopic examination. Hematoxylin and eosin were used for general staining. The alcian blue-periodic acid Schiff stain was used for mucopolysaccharides and orcein was used to stain elastic fibers.

Only minute amounts of sudan-staining lipids could be seen grossly in rabbits killed at 22 days. After 66 days sudanophilia was obvious. The deposits were localized about the orifices of the branches leading from the aortic arch. A few foamy macrophages were seen lying between the endothelium and the internal elastic membrane. No focal deposits of acid mucopolysaccharide were visible either in or near the lesions. Alcian-blue-staining material was seen in the media of both experimental and control animals. The elastic lamellae were intact.

By 94 days the lesions stained with sudan were more frequent and larger and extended from the arch to the distal thoracic aorta. With a still longer time interval the abdominal aorta became involved. Foamy macrophages, visible microscopically, caused marked thickening of the intima. Below the intimal plaques the foamy macrophages infiltrated the portion of the media near the surface, and mucopolysaccharide appeared to be increased locally causing a widening of the interlamellar spaces.

The 112 and 132 day old lesions were reorganized morphologically. Both intra- and extracellular lipid was seen located mostly in the depths of the plaques, while fibrous and elastic tissue comprised the superficial zone. Small amounts of mucopolysaccharides were also present in the depths of the lesion. Coronary arteries were severely narrowed but showed no alcian blue-staining material.

The only measurable differences between the blood chemistry of the control and experimental rabbits was an elevation of cholesterol and beta-lipoprotein in the experimental group. As both groups aged, serum mucoproteins and protein-bound hexoses rose slightly. Serum hexosamine values remained constant in both groups.

These experimental findings do not indicate that mucopolysaccharides play an essential role in the formation of atheromas in the rabbit. Only after the atheromatous process was well developed was intimal mucopolysaccharide found. Moderate in-

creases in alcian-blue-staining material were seen in interlamellar spaces beneath well-established plaques. The mucopolysaccharide appears to be a reaction of the vessel wall to the primary deposition of lipid as judged by the time of its appearance and its localization. It must be realized, however, that other species and lesions produced in other ways might reveal a different pattern.

Summary: Atherosclerosis induced in rabbits by cholesterol feeding was followed anatomically and chemically by sacrificing rabbits at intervals over a period of 132 days.

Only lipids characterized the earliest intimal lesions. Mucopolysaccharides were found only in older lesions, chiefly in the media underlying the intimal lesion. No change was seen in serum mucopolysaccharides. Accordingly, it was concluded that intimal lipid promoted the accumulation of mucopolysaccharides in arterial walls.

DIET AND GLYCOPROTEINS

Although clinical studies of the influence of diet on the carbohydrate-containing proteins of serum (including effects of fasting and feeding of carbohydrate, protein or fat to normal individuals) have proved negative (Z. Stary, *Clin. Chem.* **3,** *557* (*1957*)), rats have been found to respond to starvation (M. R. Shetlar and C. L. Shetlar, *Proc. Soc. Exp. Biol. Med.* **88,** *622* (*1955*); H. E. Weimer and H. Nishihara, *Ibid* **95,** *677* (*1957*)) and protein depletion (H. E. Weimer and H. Nishihara, *loc. cit.*) with a decrease in the serum glycoprotein.

H. E. Weimer and H. Nishihara (*J. Nutrition* **67,** *137* (*1959*)), have followed up this lead by exploiting the depletion-repletion technique of R. W, Wissler, R. L. Woolridge, C. H. Steffee, Jr. and P. R. Cannon, (*J. Immunol.* **52,** *267* (*1946*)). The animals were fed a protein-free diet until they had lost 25 per cent of their original weight. For repletion the diets contained 10 per cent vegetable oil, 4 per cent salt mixture (U.S.P. XIV) and a vitamin mixture, with the remaining 86 per cent made up of casein and starch. The percentages of protein varied as follows: 0, 8, 17, 27, 40, and 64 per cent.

Total serum glycoprotein, glycoprotein polysaccharide, seromucoid protein and seromucoid polysaccharide were determined. Albumin and globulin proteins were also measured with respect to amounts of protein and polysaccharide. Hematocrits and total hemoglobin were determined.

Hemoglobin and hematocrit values were in the normal range following depletion, but there was a pronounced hemodilution during repletion. This observation is in accord with those of E. R. Peo, Jr. *et al.* (*J. Nutrition* **62,** *475* (*1957*)). The authors point out, however, that the relative concentration changes of the serum components were of such magnitude that in only a few cases were the results masked by the fluctuations in plasma volume.

As far as weight repletion was concerned, the 40 per cent protein diet presented the best balance as shown by a more rapid repletion time and a higher food efficiency ratio even when compared to the 64 per cent protein diet.

Protein depletion caused significant reductions in all of the serum glycoproteins with the exception of the polysaccharide component of the albumin fraction. Following repletion on the 8 per cent protein diet, there was some increase in the globulin polysaccharide, but decreases were now observed in the seromucoid and albumin fractions. The 17 per cent protein diet restored total glycoprotein, albumin and globulin polysaccaride values to the normal

levels, but there was no effect on the seromucoid fraction, which remained low.

Repletion with the 27, 40 and 64 per cent casein diets all elicited comparable changes in the serum glycoprotein pattern. Total glycoprotein and globulin polysaccharide values returned to normal, but, although seromucoid hexose concentrations were elevated somewhat over those of rats fed lower levels of protein, they were lower than normal. Albumin polysaccharides were also significantly lower than normal.

Certain deductions may also be made as to the influence of dietary carbohydrate on concentrations of serum glycoproteins. The amount of starch in the repletion diets varied from 22 to 78 per cent. It was apparent that in diets which were adequate for the restoration of carcass weight, the concentration of the protein-bound carbohydrates of serum did not parallel the level of starch in the diet.

Decreases of the protein fractions of serum occurred during depletion. Albumin was decreased but globulin was decreased even further so that the albumin-globulin ratio was increased. A decrease of seromucoid protein occurred as well as of seromucoid polysaccharide. With the exception of the 8 per cent level of protein, all other repletion diets were adequate for the restoration of the major components of the serum proteins. A higher level of dietary protein, 27 per cent, was required to restore the protein moiety of the seromucoid fraction to normal. Beginning with the 17 per cent protein diet, the total serum proteins were elevated even above those of normal controls. This was due primarily to an increase in globulins, a phenomenon reviewed by J. B. Allison (*Physiol. Revs.* **35,** *664 (1955)*). Thus it can be seen that a significant decline in the polysaccharide-protein ratio occurred in all experimental groups.

The corresponding ratio for the albumin fraction was in the normal range irrespective of the nutritional state. Significant differential responses also occurred in the carbohydrate-rich seromucoid fraction during repletion.

According to the authors, these results provide evidence for the hypothesis that different physiologic mechanisms may be involved in the metabolism of the carbohydrate-rich and carbohydrate-poor proteins of serum (H. E. Weimer and J. Redlich-Moshin, *Proc. Soc. Exp. Biol. Med.* **84,** *34 (1953)*). The authors list the following as possible factors which might be responsible for the observed variances: (1) greater utilization of the protein-bound carbohydrates for cellular regeneration, (2) suboptimal amounts of a nutritional factor in purified diets necessary for the synthesis of protein-bound carbohydrates, (3) utilization of carbohydrate-rich proteins of serum, (4) slower rate of synthesis, and (5) slower release from the site of synthesis.

RELATION OF VITAMIN E AND PROTEIN TO INCISOR PIGMENTATION

Vitamin E deficiency in the rat has been shown to prevent formation of the yellow-orange pigment in the enamel of those portions of the continuously erupting rat incisors which were forming during the period of deprivation (J. T. Irving, *Nature* **150,** *122 (1942)*). Although this pigment contains iron, the addition of various levels of iron salts to a vitamin E-deficient diet did not reverse the influence of the deprivation of vitamin E (H. Granados and H. Dam, *J. Dent. Res.* **26,** *471 (1947)*).

The comparative curative effects of α-tocopherol and of protein upon the incisor teeth of vitamin E-deficient rats were appraised by J. T. Irving (*J. Dent. Res.* **37,** *732 (1958)*). Four large groups of rats were maintained in this experiment. The first three groups were fed a low-protein vitamin E-deficient diet for either 30 or 40 days, at

which time the incisors were completely devoid of the normal pigmentation and appeared uniformly white.

At this time, some of the 66 rats in group one were sacrificed for histological studies and the remainder continued for additional periods up to a total of 120 days on the vitamin E-free diet. The 60 rats in group two were supplemented with 3 mg. of α-tocopherol for additional periods from ten to 80 days. The 30 rats in group three were transferred to a vitamin E-deficient diet that had been increased to contain 18.8 per cent protein, instead of 9.3 per cent, by the addition of egg albumin at the expense of starch. These rats were sacrificed after 40 to 80 days on the new dietary regimen.

The 30 rats in group four were maintained throughout periods of 20, 30 or 40 days on the low-protein diet supplemented with 3 mg. of α-tocopherol daily. None of these control rats displayed any deficiency in ability to form the incisor pigment, either grossly or upon histologic examination. The ameloblasts contained yellow translucent granules which were high in iron and which were probably precursors of the yellow pigment of the enamel. In addition, the blood of the controls gave negative results for hemolysis by the dialuric acid test which has been proposed for detection of vitamin E-deficiency by P. György and C. S. Rose (*Ann. N. Y. Acad. Sci.* **52,** *231 (1949)*).

In the rats in group one, the enamel organ showed the typical degenerative changes characteristic of vitamin E deficiency; fibrous tissue containing many macrophages with large amounts of iron-rich pigment replaced the enamel organ. In the ameloblasts which had already formed in older areas of the enamel organ, there was a progressive decrease in the number of yellow iron-containing granules until almost a complete absence was noted after 30 days. The dialuric acid test was positive at the same time.

The first response of the rats in group two which were provided with α-tocopherol was the reappearance of granules in the ameloblasts at about ten days. In the early stages, the iron-containing cells were infrequent but as the enamel organ recovered with a reduction in macrophages and fibrous tissue, more iron-containing ameloblasts appeared. Complete recovery was not noted by 80 days in some cases, even though the incisor had replaced itself twice completely in that period. The dialuric acid test was faintly positive after ten days of therapy but was consistently negative thereafter.

The results with incisors of the rats in group three with protein supplementation instead of vitamin E were virtually the same as those in group two, both visually and upon histologic examination. The yellow pigment began to appear grossly in the enamel after 29 to 64 days of supplementation. However, throughout the trial periods the dialuric acid test for hemolysis remained positive, indicating that the vitamin E-deficient influence upon the fragility of red blood cells had not been relieved by protein supplementation even though the normal formation of incisor pigmentation had been reinstated.

The interrelationship of vitamin E and protein in their influence upon the formation of the incisor pigment is an interesting one that needs further exploration. This phenomenon is the more interesting because of the inability of added protein to relieve other evidences of vitamin E deficiency.

VITAMIN A DEFICIENCY AND ORAL CARCINOGENESIS

Oral cancer, including cancer of the lip, is said to constitute 8 to 10 per cent of all cancer observed in the United States and to be responsible for 4 per cent of the deaths

attributable to cancer (S. L. Lane, *Oral Surg. Oral Med. Oral Path.* **6,** *258 (1953)*). Yet the study of cancer production in the oral cavity has been limited to relatively few trials, with experimentally produced cancers reported by J. J. Salley (*J. Dent. Res.* **33,** *253 (1954)*).

In the belief that the clinical and histologic changes observed in avitaminosis A and in some precancerous lesions had some basic similarities, N. H. Rowe and R. J. Gorlin (*J. Dent. Res.* **38,** *72 (1959)*) conducted a study to explore the possibility of an interrelationship in hamsters. The diet used was a rather unusual one prepared to order in the form of bread containing 6 ounces of sugar, 4 ounces of salt, 8 ounces of lard, 4 ounces of yeast, 1 gallon of water, 4 pounds of whole wheat flour and 8 pounds of white flour. All 120 hamsters in the experiment were maintained on the diet for 20 weeks beginning at 45 days of age. The first group of 40 hamsters received only the deficient diet ad libitum. The second group of 30 hamsters received the same diet ad libitum and a supplement of 400 I.U. of vitamin A once each week. The third group of 30 hamsters received the same supplement and the vitamin A deficient diet in amounts equivalent to what was consumed by the subjects in group one. Each hamster in these three groups received twice per week applications of a 0.5 per cent solution of 7,12-dimethyl-1,2-benzanthracene in liquid petrolatum heavy grade to the left buccal cheek pouch for the first 13 weeks of the experiment.

The ten hamsters in each of groups four and five were identically fed to those in groups one and two; however, applications of the liquid petrolatum without the carcinogen were made at the same intervals. For seven weeks after the carcinogen and petrolatum applications were made, the hamsters were continued on the same dietary regimens.

After five weeks on the deficient diet, symptoms of mild vitamin A deficiency began to appear in the unsupplemented groups when the fur lost its characteristic smoothness and became slightly ruffled in appearance. The fur changes became marked by seven to eight weeks. Very slight xerophthalmia was evident by nine weeks and weight losses began after 11 weeks. The hamsters fed unrestricted amounts of the deficient diets and the vitamin A supplement did not grow normally, gaining a total of only 6.3 g. on the average per hamster in contrast to a 4.5 g. loss for the deficient animals (group one) and a 4.0 g. loss for those restricted in food intake (group three). The poor growth exhibited by the unrestricted controls suggests that the supplemented diet was still not adequate for the nutriture of this species. The serum of the deficient hamsters contained 19 μg. per cent of vitamin A, while the serum of the supplemented animals five hours after the vitamin administration contained 57 μg. per cent of vitamin A and only 26 μg. per cent a week later.

At the end of the experimental period, the hamsters were sacrificed and the cheek pouches examined grossly and in histologic preparations. On microscopic examination, the mucosa of the pouch was graded into six categories: (1) normal, (2) vitamin A deficient, (3) hyperkeratotic, (4) benign papilloma, (5) carcinoma in situ, and (6) invasive squamous-cell carcinoma.

The incidence of clinically observable tumors was greater in the unsupplemented group one than in either of the supplemented groups two and three. No lesions were observed in groups four and five. Among the tumors clinically observed in 22 of the 27 deficient hamsters, carcinoma in situ or invasive squamous-cell carcinoma were observed in 16 (59 per cent). Among the 27 vitamin A supplemented hamsters, 20 had tumors of all types, of which nine (33 per cent) had malignant types. Among the 12 vitamin A supplemented, calorie-restricted hamsters, seven had tumors and three (25 per cent) had malignancies. The

epithelia of all hamsters in groups four and five were histologically considered to be normal.

These data suggest that vitamin A deficiency increased the susceptibility of hamsters to carcinogen-induced benign and malignant tumors. It is unfortunate that these studies were not conducted with a diet clearly deficient in vitamin A without the suspicion of other simultaneous deficiencies, and that more frequent vitamin A supplementation in greater amounts was not given to prevent the gross variations in serum vitamin A.

PYRIDOXINE DEFICIENCY IN MICE

Pyridoxine deficiency in the rat and many other animals is characterized by impaired growth of young animals, seborrheic skin lesions and nervous disorders (*Present Knowledge in Nutrition*, The Nutrition Foundation, New York, 1956). In certain animals, as dogs and swine, it is also associated with a microcytic hypochromic anemia (*Nutrition Reviews* **3,** *29* (*1945*)). Development of an anemia has also been shown to result from exclusion of pyridoxine from the diet of mice (H. P. Morris, T. B. Dunn and B. P. Wagner, *J. Nat. Cancer Inst.* **14,** *493* (*1953*)) and men (*Nutrition Reviews* **17,** *127*, (*1959*)). Anemias also result from deficiencies of folacin and vitamin B_{12}.

In a study designed to characterize the pyridoxine deficiency disease in mice and the effect on it of parenterally administered pyridoxine, folacin and vitamin B_{12}, L. Mirone and C. D. Jackson (*J. Nutrition* **67,** *167* (*1959*)) divided 300 weanling mice into three groups of 30, 30, and 240. They then fed them respectively, an adequate diet, the same diet without pyridoxine and the pyridoxine-free diet with 25 mg. per kg. of deoxypyridoxine, a pyridoxine antagonist. After five days, the deoxypyridoxine was omitted from the third diet.

Both groups of mice on the pyridoxine-deficient diet developed the same symptoms. Their average weight was about 10 g. less than that of the controls. Many developed a bloody diarrhea, thinning of the hair, paralysis of the hind legs and tremors. The symptoms appeared about two weeks earlier in the mice fed deoxypyridoxine, and 44 per cent of these animals died during the first 12 days of the experiment. Hematological studies made just before vitamin supplementation revealed that in the deficient mice there had been decreases of 38, 35 and 52 per cent in the hemoglobin, erythrocyte and leucocyte levels, respectively. There had been a decrease in mononuclear cells and an increase in polynuclear cells.

After 20 days of daily injections of 50 micrograms of pyridoxine, one group of mice had regained normal weight and no longer had symptoms of pyridoxine deficiency. This was also the case in groups which were given injections of pyridoxine in combination with 0.4 micrograms of vitamins B_{12} or 5 micrograms of folacin daily. In the absence of pyridoxine, however, the B_{12} and folacin supplements, either alone or in combination, had little or no effect and the animals receiving these vitamins were little better off than those receiving no supplementations. Most of these succumbed during the period of treatment, as did all of those receiving either a saline injection alone or no injection.

Thus it is evident that vitamin B_{12}, which is effective in pernicious anemia, and folacin, which is effective in other macrocytic anemias, have no beneficial value in the hypochromic microcytic anemia of pyridoxine deficiency.

NOTES

Protein-Calorie Malnutrition in Pre-School Children

D. B. Jelliffe (*J. Pediat.* **54,** *227 (1959)*) has reviewed the current knowledge concerning kwashiorkor. His review not only covers the recent literature, but highlights what is known concerning protein malnutrition and reviews the various approaches toward solution of this problem in several parts of the world.

The 168 references constitute a fairly complete recent bibliography of the subject. This review is too condensed for further abstraction, and it is of sufficient value to be called to the attention of readers of *Nutrition Reviews.*

Food Standards

Many nutrition scientists will be interested to know that substantial progress is being made in working toward greater uniformity in food and drug legal standards and regulations. The Food Law Institute, Inc., 608 Fifth Avenue, New York 20, N. Y., has sponsored translations of a drafted uniform code adopted by the Latin-American Chemical Congress, as prepared by the committee of which Dr. Carlos A. Grau of Argentina is chairman.

Plans are underway, also, to translate and publish a drafted uniform code (and a digest of the code) prepared by representatives of the European Common Market countries. Mr. Charles Wesley Dunn, President of the Food Law Institute, has been a notable leader in working toward this very important objective.

Also available from the Food Law Institute is a booklet giving an account of the organization's ten year record.

Fifth International Congress on Nutrition

Plans for the Fifth International Congress on Nutrition to be held in Washington, D. C., September 1 through 7, 1960, are now being finalized. Organized by the American Institute of Nutrition and the U.S.A. National Committee for Nutritional Sciences of the National Academy of Sciences, the Congress is under the auspices of the International Union of Nutritional Sciences. Officers are as follows: Honorary President, Elmer V. McCollum; President, C. Glen King; Chairman, Organizing Committee, Paul György; General Secretary, Milton O. Lee.

Eminent nutritional scientists from all parts of the world will participate in the Congress. The scientific program will consist of:

A. Panel discussions by invited participants on:
- (1) Evaluation of Nutritional Status in Man
- (2) Effects of Processing and Additives on the Nutritive Values of Foods
- (3) Lipids in Health and Disease
- (4) Animal Nutrition and Food Production
- (5) Nutrition in Maternal and Infant Feeding
- (6) Proteins and Amino Acids in Nutrition
- (7) Three Hours Around the World; New Possibilities in Nutrition Research

B. Special Programs on:
- (1) Preservation of Foods by Ionizing Radiation
- (2) Metabolism of Fission Products
- (3) Use of Radioisotopes in Nutrition Research
- (4) Nutritional Physiology of Ruminants
- (5) New Aspects of Carbohydrate Metabolism
- (6) Micro-nutrients in Plant and Animal Nutrition
- (7) Functional Role of Vitamins
- (8) Human Nutritional Requirements and Allowances
- (9) Nutrition of Cells and Tissue Culture

C. An all-day symposium on Food Needs and Food Resources, presented by invited authorities, centered upon:

(1) Food Problems of the World's Increasing Population
(2) Soil and Water Resources
(3) Increasing World Production and Distribution of Food
(4) Major Nutrition Problems Today
(5) Socio-Economic Factors That Limit Needed Production and Consumption
(6) The Prospect of Meeting Protein Needs
(7) Food and Protection of Public Health

D. In addition, ten-minute papers reporting original research will be accepted to the limit of facilities available.

There will also be scientific and industrial exhibits, visits to various scientific laboratories, a special program for women guests, and general entertainment activities for all registrants.

The final announcement of the Congress will be issued in November, 1959. To be placed on the mailing list, or for additional information, write: The Secretariat, Fifth International Congress on Nutrition, 9650 Wisconsin Avenue, Washington 14, D.C.

Problems of Human Nutrition

Some 60 pages of a recent issue of the *American Journal of Clinical Nutrition* (**6,** *459 (1958)*) are devoted to the papers presented at a Symposium on Problems of Human Nutrition sponsored by the Medical College of Georgia and the National Vitamin Foundation, Inc., in honor of Dr. V. P. Sydenstricker. These papers summarize the thoughts of a group of eminent nutritionists on a number of subjects that are of both practical and fundamental significance.

In his discussion of human deficiency disease syndromes, R. H. Follis, Jr., emphasizes the paucity of information concerning the pathogenesis of deficiency diseases using as examples kwashiorkor, goiter and beriberi; the subject of kwashiorkor is further developed by Joaquin Cravioto. The role of antibiotics in nutrition and the interrelationships among nutrition, metabolism and infection are discussed by Paul György. Problems of importance in the use of intravenous fat alimentation are summarized by J. F. Mueller. The knowledge of niacin-tryptophan relationships in man is reviewed by Grace Goldsmith. Some of the problems in the interpretation of studies on amino acid imbalance in nutrition are appraised by W. D. Salmon. The subject of lipotropic factors is reviewed by C. C. Lucas with emphasis on the separation of the facts from the fantasies associated with this topic. The symposium concludes with a review of the subject of vitamin B_{12} deficiency in untreated patients with pernicious anemia by W. J. Darby and associates.

The publication of this series of papers constitutes a real service to those unable to attend the symposium. The papers provide a great deal of pertinent information on the subjects under discussion, but, even more, they should provide a source of stimulation for those concerned with either clinical or animal nutrition studies.

Recent Books

Food Values in Shares and Weights (in common servings with suggestions for personal food needs). Second Edition. Clara Mae Taylor, Ph.D. The Macmillan Company, 60 Fifth Avenue, New York. Price $4.00. Pp. 116.

Rowett Research Institute's Collected Papers, Vol. XV. 1959, and a separate *Summary and Subject Reviews*, pp. 50. The Rowett Research Institute, Bucksburn, Aberdeenshire, Scotland.

Proteins in Foods. S. Kuppuswamy, M. Srinivasan and V. Subrahmanyan. Special Report Series No. 33, Indian Council of Medical Research, New Delhi, 1958. Printed in India by K. A. Korula at the Wesley Press, Mysore City. Price Rs. 12. Pp. 28.

Handbook of Diet Therapy. Dorothea F. Turner. University of Chicago Press. Price $5.00. Pp. 208.

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The Nutrition Foundation was organized by food and related manufacturers in December, 1941, as a sincere expression of their interest in scientific progress and human health. The basic purposes of the Foundation are: (1) the development of a comprehensive program of fundamental research, providing basic information in the science of nutrition; and (2) the support of educational measures that will assist in making the science of nutrition effective in the lives of present and future generations.

The publication of NUTRITION REVIEWS has been undertaken to enable professionally trained people to keep abreast of current progress and to have available an unbiased, authoritative review of the current research literature in the science of nutrition.

The Editorial Staff has been carefully selected so that the publication will merit, in reasonable degree, the confidence of the medical profession and others who work directly with the public in the field of nutrition. The service provided by the publication is distinct from that of an abstract journal, a review for laymen, or an extensive scientific review. Since the reviews as originally prepared by a member of the Editorial Staff are frequently modified by special referees and may be further modified by the Editorial Office in preparing final copy for the press, the Editorial Committee feels that it is preferable not to affix the names of Editorial Staff members to individual reviews. There is thus a high degree of group responsibility on the part of the Editor and Associate Editors.

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